Synaptic plasticity: **Fastening synapses by adhesion**

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New genetic studies have revealed that synaptic plasticity at the *Drosophila* neuromuscular junction can be dissociated into an adhesion-molecule-based pathway responsible for structural changes and a cAMP signaling pathway required for functional changes.

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Electronic identifier: 0960-9822-007-R0028

Current Biology 1997, 7:R28–R30

The initial formation of synaptic contacts in the nervous system is often followed by periods of synaptic rearrangement, when synapses may grow or regress and be eliminated. Such synaptic rearrangements allow the nervous system to adapt as it develops, increasing the size and strength of a synapse as the postsynaptic cell grows, or in other cases refining synaptic connectivity by the elimination of selected inputs. Less extreme structural changes may also accompany the processes of learning and memory. Given that a synapse involves the physical attachment of the presynaptic terminal to the postsynaptic cell, it was perhaps not surprising that adhesion molecules would be found to play a role at some step in the process of synapse formation and subsequent growth and/or regression [1].

In the sea slug *Aplysia*, behaviorally relevant long-term alterations in synaptic strength have been shown to involve the addition or regression of synaptic contacts, or boutons. These changes were correlated with alterations in the levels of presynaptic or postsynaptic ApCAM, a membrane protein related to the 'neural cell adhesion molecule' NCAM [2,3]. A recent series of papers [4–6] has now reported studies that exploit the detailed knowledge of the *Drosophila* larval neuromuscular system [7] and the power of genetic manipulation in this organism to show that Fasciclin II (Fas II), a relative of vertebrate NCAM, plays a critical role in the developmental regulation of synaptic size.

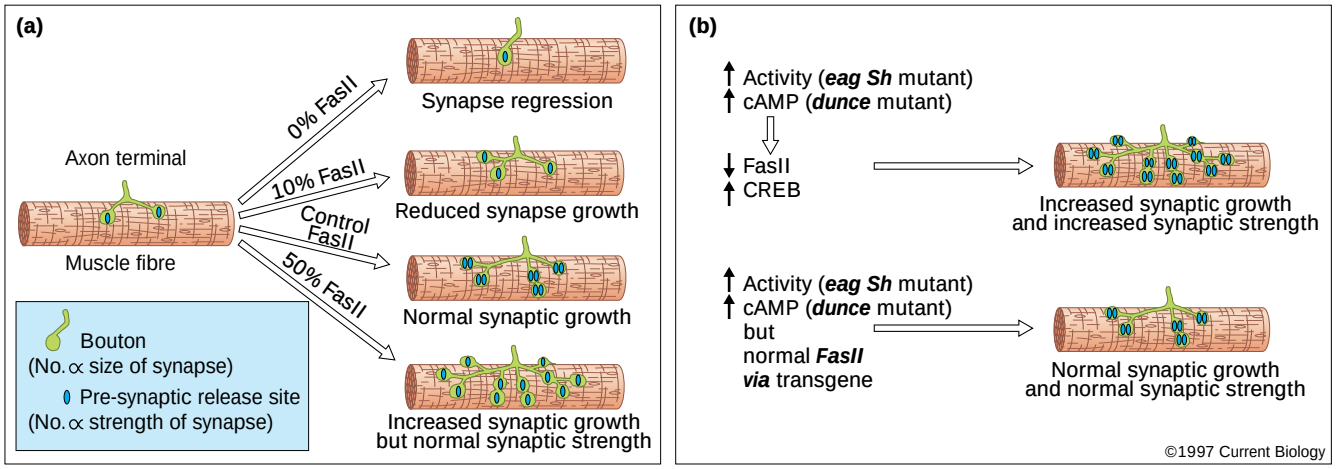
Interestingly, these new studies also provide support for the idea that the processes of developmental synaptic plasticity and of learning and memory may share at least some common cellular mechanisms. A previously characterized *Drosophila* mutation, *dunce*, known both to affect learning and to result in an increased number of synaptic boutons at the larval neuromuscular junction [8], has been shown to affect synaptic size by altering the level of Fas II. And the new work has also revealed a parallel, Fas II-independent pathway that involves cyclic AMP (cAMP) signaling and is required to bring about changes in the functional strength of synapses to accompany the changes in synaptic size produced by the Fas II-dependent pathway.

Although Fas II is expressed by both motor axons and their target muscles during axon outgrowth and synapse formation, axon outgrowth and initial synapse formation were found to be normal in mutant flies totally lacking Fas II [4]. However, not only did these synapses fail to exhibit the normal increase in bouton number during the next few developmental stages, but they actually regressed. The mutants became sluggish and died by the first larval instar. Schuster *et al.* [4] were able to rescue the mutant flies from death by using a genetic method — crossing a specific GAL4 enhancer-detection line of flies with *Fas II* null mutants containing a *UAS–Fas II* transgene — to drive different levels of Fas II expression in specific subsets of motor neurons and muscle fibers.

Rescue experiments of this kind enabled Schuster *et al.* [4] to show that the normal increase in the number of synaptic boutons requires normal levels of Fas II in both the motor neuron and the muscle fiber. In mutants that expressed only 10 % of control Fas II levels, the increase in bouton number was less than in normal flies. More unexpected was the finding that, in flies expressing 50 % of the wild-type level of Fas II, the number of synaptic boutons was increased to almost double the control value. This study [4] thus not only showed that Fas II expression is required for the normal growth of synaptic contacts, but also revealed a complex relationship between Fas II levels and normal synaptic growth, with a fairly narrow optimum (Fig. 1a).

This finding is interesting as it suggests that downregulation of adhesion molecule expression at the synapse may be required for the sprouting of new synaptic boutons. This is reminiscent of the situation in *Aplysia*, where downregulation of presynaptic ApCAM was associated with the sprouting of new synaptic boutons during a behaviorally relevant change in synaptic strength [2]. Enlarged synapses, like those in the 50 % Fas II flies, were previously observed in two other fly mutants — *dunce* and a double mutant of *ether a go go* and *Shaker* (*eag Sh*). In these mutants, an increase in cAMP levels (*dunce*), or an increase in neuromuscular activity that leads to an increase in cAMP levels (*eag Sh*), resulted in synapses with an increased number of terminal branches and boutons [8]. These synaptic abnormalities were suppressed when a transgene was used to maintain Fas II at normal levels [5].

Figure 1



(a) Genetically altering the amount of the NCAM-like adhesion molecule Fas II affects the structural growth of the synapse, and depending on the level can result in either synaptic regression or the formation of larger than normal synapses. However, these stucturally larger synapses are not functionally stronger than normal. (b) Changes in the functional strength of the synapse – the amount of transmitter actually released from the nerve terminal – are caused by a separate activity-dependent pathway that involves cAMP signaling and CREB activation. However, this increase in the amount of transmitter relased only occurs when the synapse has been enlarged by the Fas II-dependent pathway.

Thus Fas II was shown to be both necessary [5] and sufficient [4] for these activity-dependent changes in synaptic size at the neuromuscular junction.

The increase in synaptic size produced by reducing Fas II levels was not, however, accompanied by an increase in the functional strength of the synapse. Even though there were twice as many synaptic boutons, each bouton was shown to release only half the normal amount of transmitter. The overall functional strength of each synapse was thus not different from normal. It appeared as if the control number of presynaptic release sites had simply been distributed among the increased number of boutons. This observation fits with the results of a recent study [9] of the assembly and localization of presynaptic active zones — sites of transmitter release — in this system. Each motor neuron appeared to produce a characteristic number of such sites, which normally become localized to the synaptic boutons. However, when the number of boutons was reduced by reducing the number of mature muscle fibers with other mutations, presynaptic release sites were still made in apparently normal numbers. Without sufficient boutons as targets, many of these release sites became distributed along the length of the presynaptic axon.

In contrast to the enlarged synapses in the 50 % Fas II mutants, the activity-dependent increase in synaptic size produced in the *dunce* and *eag Sh* mutants is accompanied by an increase in the amount of transmitter released [8]. Thus, these mutations increase synaptic size by altering the level of an adhesion molecule, Fas II, and increase synaptic strength *via* a Fas II-independent pathway. As both *dunce* and *eag Sh* are known to cause an increase in the levels of cAMP [8], potential downstream targets were investigated by altering the activity of CREB, the 'cAMP-response-element-binding protein' [6]. This signaling pathway was blocked downstream of the step affected by the *dunce* mutation by causing the expression of a CREB repressor, dCREB-2. This prevented the increase in synaptic strength that occurs in *dunce* mutants, but not the increase in synaptic size. Conversely, expression of a CREB activator increased synaptic strength, but only in those *Fas II* mutants where the number of boutons was increased. Thus, these papers [4–6] together show that, at the *Drosophila* neuromuscular junction, cAMP initiates parallel changes in CREB and in Fas II that result in long-term alterations in synaptic structure and function (Fig. 1b). Blocking the CREB-mediated pathway has recently been found to block long-term memory in *Drosophila* [10] and long-term synaptic plasticity in *Aplysia* [11].

While many additional processes will no doubt be shown to affect synaptic plasticity (see, for example, [12]), these new studies are intriguing in that they highlight a number of similarities in the process of synaptic plasticity in *Drosophila* and *Aplysia*, and suggest that downregulation of an NCAM-like molecule is required for the sprouting of new synaptic contacts during activity-dependent remodeling of synapses. The extent to which such processes are required during learning, especially in vertebrates, is not yet clear. Nevertheless, it has recently been found that

NCAM-deficient mice have learning deficits, and a recent paper [13] provides evidence that this is because they lack the polysialic acid carried by NCAM. Intriguingly, polysialic acid acts to reduce adhesion, and its expression is activity-dependent [14]; it may therefore play the same role as that played by Fas II or ApCAM downregulation in synaptic plasticity in *Drosophila* or *Aplysia*, respectively. In any case, it is clear that using a combination of genetic and cell biological approaches to dissect out the cellular mechanisms underlying developmental synaptic plasticity will help to reveal the extent to which similar processes are involved in learning and memory.

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